PATENT
Attorney Docket No.: 018941-000710US
Client Ref. No.: B99-017-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Lemaux, *et al.*

Application No.: 09/384,811

Filed: August 27, 1999

For: TRANSPOSON TAGGING AND GENE DELIVERY IN SMALL GRAIN CEREALS

Examiner: Cynthia Collins

Art Unit: 1638

DECLARATION UNDER 37 C.F.R. §1.132 BY PEGGY LEMAUX, Ph.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

1. I, Peggy Lemaux, Ph.D., am a Cooperative Extension Specialist at the University of California, Berkeley and am currently the Director of the University of California Division of Agriculture and Natural Resources Biotechnology Workgroup. I am a co-inventor of the subject matter of the above-referenced patent application. I have worked in the field of plant genetics for over 15 years.

2. I hold a Ph.D. from the University of Michigan, which was conferred in 1977. I have authored over 65 publications relating to microbial and plant genetics and molecular biology. A copy of my curriculum vitae is attached as Exhibit 1.

3. I have read and am familiar with the contents of the above-referenced patent application and claimed subject matter. It is my understanding that the Examiner has rejected the claims as allegedly unpatentable over the prior art. In particular, the Examiner believes that the invention is obvious over the combination of various prior art publications, of which the primary references are McElroy *et al.* "Development of a simple transient assay for *Ac/Ds* activity in cells of intact barley tissue" *The Plant Journal* 11:157-165, 1997), Wan *et al.* ("Generation of Large Numbers of Independently Transformed Fertile Barley Plants" *Plant Physiol.* 104:37-48, 1994), and Bancroft *et al.* ("Development of an efficient two-element transposon tagging system in *Arabidopsis thaliana*" *Mol. Gen. Genet.* 233:449-461, 1992). The Examiner alleges that the success of Wan *et al.* in transforming barley, the success of McElroy *et al.* in demonstrating Ac transposase-mediated excision of *Ds* in barley cells, and the use of the *Ac/Ds* transposase for stable transformations in a number of other systems, *e.g., Arabidopsis*, would lead one in the art to use the system in barley with a reasonable expectation that it would work in barley.

4. This declaration is submitted to provide further evidence that prior to Applicants' invention, one of skill could not reasonably expect the *Ac/Ds* system to generate stable transformants in barley, *i.e.,* transformants in which the transposable element can be reactivated and reinsert into the genome, because of certain characteristics of the barley cell and genome, *e.g.*, the amount of methylation and gene silencing.

5. The claims under prosecution are drawn to plants containing an *Ac* or *Ds* transposon stably integrated into the genome and methods of transforming plants using the *Ac/Ds* system. The transposons in the claimed barley plants can be excised and reintegrate into the genome in the presence of a transposase enzyme. Although stable integration in barley plants has been obtained using other transformation systems, such as that taught by Wan *et al.*, it has been very difficult for those in the art to generate barley

transformation systems based on the *Ac/Ds* system, even though it has been used in many other plants. In order for the *Ac/Ds* system to provide usable stable transformants in barley, the elements must be introduced into plants and stably integrate into the genome. That is, they must not be subject to rearrangement, deletions, etc. over time. Further, they must retain their ability to transpose *i.e.,* the transposase must not be silenced and the recognition sites not be methylated or changed in sequence; and the *Ds* elements must retain the ability to be re-insert into the genome.

6. Prior to our invention, it was unknown whether such conditions could be met in barley. Instability of gene expression and gene silencing are believed to be due, at least in part, to methylation. The barley genome is known to be highly methylated. It was not known whether the architecture of the highly methylated genome would permit high levels of re-insertion of transposons. Further, as explained below, frequent methylation of foreign sequences has been demonstrated in barley and has been shown to frequently lead to instability and/or gene silencing. Accordingly, one could not predict whether the methylation status of barley would provide for stable, active *Ac/Ds* integration and re-integration.

Instability of foreign sequences in barley

7. McElroy *et al.* teach a transient, transgene introduction system that shows that a *Ds* transposable element can be excised from a reporter plasmid in barley cells in the presence of *Ac* transposase activity from an Ac transposase gene introduced via bombardment at the same time. However, transposable elements can undergo deletions, internal rearrangements and/or methylation-mediated inactivation converting an active, movable element into an element incapable of movement. Critically, the reference is silent on whether the excised *Ds* elements could re-integrate into the barley genome, a feature vital for effective transposon tagging or gene delivery.

8. It has long been known that barley is highly methylated and that methylation plays a role in instability of foreign sequences in barley. In an exemplary study, Rogers & Rogers (*Plant Mol. Biol.* 18:945-961, 1992, submitted herewith as Exhibit 2) tested the effect of methylation of foreign DNA on its stability after introduction into barley plants. The investigators noted that foreign DNA could be introduced into barley cells. Further the foreign DNA persisted through at least two plant generations. However, they pointed out that this persistence was not equivalent to stable transformation and that the DNA was frequently rearranged or lost in subsequent generations. They attributed the instability, at least in part, to methylation. They further noted that cereals have an unusual genome organization where structural gene sequences are very GC-rich. Methylation systems that are endogenous to barley are in part dependent on the GC-rich nature of the genome. Foreign sequences, however, do not share this feature. They demonstrated that foreign sequences were methylated in a specific pattern that was distinguishable from the methylation pattern of the highly GC-rich barley sequences and concluded that the foreign sequences could therefore be easily distinguished from endogenous sequences and thus preferentially inactivated.

Loss of activity in barley

9. Inactivation of foreign sequences by methylation may lead not only to direct instability and loss of a sequence from the genome, but may also lead to lack of activity of the encoded protein or compromise the ability of the introduced sequence to perform its function. Gene silencing is thought by those in the art to be due to methylation of regulatory sequences or, in some cases, the coding sequences of genes. Thus, methylation may silence expression of a gene, for example, an *Ac* transposase gene or a transposition cassette associated with a *Ds* element. Further, methylation can lead to direct inactivation of transposition, for example, by preventing a *Ds* element from stably integrating and retaining the ability to reinsert into another region of the genome in the presence of transposase activity. Lastly, it is also believed that the inverted repeats in the

Ds elements, needed for recognition by *Ac* transposase and transposition, can trigger methylation-induced silencing that prevents re-activation of *Ds*. Thus, methylation in barley could also result in gene silencing.

10. Not only is the ability of the *Ds* element to re-insert important for transposon tagging and expression, it is also important in the generation of plants in which it is desirable to lose the transformation vector or selectable marker sequences. The latter capability can be used to obtain integration of a transgene contained within *Ds* inverted repeat ends at a position unlinked to the site at which the transformation vector originally integrated. This means that in subsequent generations, plants may be obtained that contain only the *Ds* inverted repeat ends and the inserted transgene of interest and not the other nucleic acid sequences contained in the original transformation vector. Retaining the activity of the transgene of interest and its ability to relocate under the control of *Ac* transposase requires that expression persist through multiple generations.

Conclusion

11. Thus, even though McElroy *et al.* showed in a transient assay system that *Ac* transposase is active in barley and can excise a *Ds* cassette bearing the inverted repeats and a transgene of interest from plasmids that are transiently introduced into barley, there is no teaching or suggestion that *Ds* can excise and then re-integrate in subsequent generations in the presence of transposase activity. Further, neither Wan *et al.* nor Bancroft *et al.* provide any teaching or suggestion that the *Ds* element will be able to reintegrate in the highly methylated barley genome or will not itself become methylated and incapable of excising or reintegrating in a stably transformed barley cell.

12. Therefore, for the reasons provided above, one of skill in the art, at the time the application was filed, would not have been able to use the *Ac/Ds* transposon system in barley with a reasonable expectation of success for obtaining barley plants containing an

Ac or *Ds* transposon stably integrated into the genome where the Ds element can excise and reintegrate into the genome in the presence of transposase.

13. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that +these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon.

Date: Dec. 13, 2002 By: _____
Peggy Lemaux, Ph.D.